THE EFFECT OF THE TEMPERATURE REGIMEN USED DURING CULTIVATION ON THE CHEMICAL COMPOSITION OF PASTEURELLA PESTIS

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THE EFFECT OF THE TEMPERATURE REGIMEN USED DURING CULTIVATION ON THE CHEMICAL COMPOSITION OF PAST. PESTIS

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It is known that cultivation of the plague microbe at a temperature greater than 37° leads to a change in its antigenic structure. Under these conditions the plague microbe intensively produces fraction 1 and the V- and W- antigens. These temperature dependent antigens are easily detected with the help of the precipitation reaction in gel and based on the data of numerous investigators are characteristic for virulent and immunogenic strains of plague microbe (Burrows, 1960; Surgalla, 1960; Fukui et al., 1960).

Attempts have been made to determine displacements in the chemical composition of the plague microbe which emerge following a change of its antigenic structure. Fukui et al. showed that the synthesis of fraction 1 and the VW antigens observed during incubation of the plague microbe with aeration at 37° was accompanied by an increase in the content of protein and ribonucleic acid in the cells. Bakhrakh and Yegorova (1962) reported a significant increase in the amount of polysaccharide in cultures of plague microbe cultivated on agar media in comparison with the polysaccharide content in cultures cultivated at 28°.

In the present work a comparative chemical characterization is presented of the EV vaccine strain cultivated on an agar medium under the conditions for the production of plague vaccine at 28 and 37°. The establishment of the bonds between the conditions of cultivation and the chemical composition of the plague microbe undoubtedly will help in clarification of the optimal conditions for the synthesizing of practically all the specific fractions by the plague microbe.

The culture of plague microbe was incubated in an AKMSh device at 28° for 48 hours and at 37° for 72 hours since in the latter case growth was retarded.

The incubating culture was mixed with a physiological solution of sodium chloride and the suspension was filtered through 3-4 layers of gauze (with the aim of liberating it from the particles of the agar medium). It was centrifuged at 5,000-6,000 rpm and washed off three times with a physiological solution. It must be pointed out that cells of the plague microbe incubated at 37° precipitate poorly and it was difficult to wash them out. The washed cells were separated out with cold acetone and dried in a vacuum-dryer over calcium chloride.

The resultant preparations of plague microbe, incubated at 28 and 37°, were subjected to chemical analysis. Determinations were made in the preparations of the ash content, moisture content, the amount of nitrogen (by the method of isothermic distillation in Conway dishes), phosphorus (by the Fiske-Subbarow alteration method with ascorbic acid), glucosamine (by the Elson-Morgan method with Gladyshev's modification, 1956). The polysaccharide content in the cells was established according to the reaction with anthrone reagent (Gary et al., 1957).

The various forms of phosphorus were determed by the method of Schmidt and Thannhauser (1945). Free lipids were extracted with ether in a Sokslet apparatus. The overall amount of lipids was determined after treatment of the cells with hot alcohol (Belozerskiy and Proskuryakov, 1951). Monosugars were identified by the chromatographic method and their quantity determined by the method described by Zaytseva and Afanasyeva (1957)

The results obtained showed (tables 1 and 2) that based on chemical composition the plague microbes incubated at 37° differed from the microbes incubated at 28° by a considerably increased content of polysaccharide, a greater amount of lipids and a decreased amount of glucosamine and phosphorus, mainly RNA phosphorus.

During chromatographic analysis of the polysaccharide segment of both cultures, galactose, glucose, ribose, and xylose were detected. The quantity of galactose in the cultures incubated at 37° was more than 20 times greater than in cultures of plague microbe incubated at 28°. On the contrary the content of glucose and ribose was approximately two times higher in cultures incubated at 28° (table 3).

The unusually high content of galactose in the cultures of plague microbe incubated at 37° compelled the setting up of special experiments for checking the completeness of washing off the culture from the medium

since due to the latter the content of galactose, which is the basic component of the agar of the medium, may be increased in the cells. Numerous tests showed that in reality in the supernatant liquid following centrifugation of the washout from the plague microbe cultures, there was a considerable amount of sugars, but already after two or three washings of the cells with a physiological solution, practically no polysaccharide was determined in the centrifugate. Consequently, it can be considered that as a result of a triple washing with a physiological solution the cells of plague microbe are completely or almost completely liberated from the poly saccharide of the medium which are not bound with them.

Simultaneously in separate experiments it was noticed that the amount of polysaccharide in washed cells of plague microbe incubated at 37° was considerably increased in comparison with unwashed ones. Correspondingly, the protein content in the washed cells was somewhat decreased. In the cells of cultures incubated at 28°, the amount of polysaccharide decreased in the process of washing (table 4).

It can be suggested that plague microbes cultivated at 37° accumulate polysaccharide. With this the accumulation of polysaccharide may be prolonged by nonproliferating cells following their washing off during centrifugation. At the same time it isn't ruled out that the increase of the relative content of polysaccharide in washed cells incubated at 37° may be explained by the removal during the process of washing of the surface easily soluble protein components.

Two serological tests with antiplague serum were set up with plague microbes incubated at 28 and 37°--the precipitation reaction in gel (for the specific capsule protein - fraction I) and the hemagglutination reaction (for the specific polysaccharide). It turned out that in cells of plague microbe incubated at 28°, in comparison with cells incubated at 37°, considerably more specific polysaccharide was detected and much less capsular specific protein.

Consequently, the polysaccharide accumulated by plague microbes in considerable amounts during growth at 37° is not specific and represents a reserve substance.

A similar effect was noted even earlier in several species of bacteria and yeasts in which the glycogen fluctuated from 2 up to 30% depending on the conditions of cultivation. Holme and Palmstierna (1956) and Holme (1957), while incubating intestinal bacilli on a synthetic medium, established that the accumulation of a considerable amount of polysaccharide by growing and quiescent cells was connected with a deficiency in nitrogen nourishment.

From this point of view it is possible to analyze our recording of the accumulation of a polysaccharide of the galactan type by cells of plague microbe which had been incubated at 37°. Many investigators (Hills and Spurr, 1952; Higuchi and Carlin, 1958; Brownlow and Wessman, 1960) showed that for the growth of the plague microbe under these conditons the presence is required in the nutrient medium of some additional amino acids and growth substances.

In recent years the proposition has been expressed that the synthesis of fraction I (capsular specific protein) by the plague microbe is the result not of intensified production of this antigen, but is caused by the impossibility of further utilization of it on account of obstruction of some enzyme systems at 37°. A manifestation of the specified condition in particular is the necessity at this temperature in a number of additional sources of nitrogen (Burrows, 1960).

Since the accumulation noted by us of polysaccharide by the cells of the plague microbe is also apparently connected with a deficiency in sources of nitrogen nourishment, a conclusion can be made concerning the existence of a bond between the synthesis of the capsular protein and the accumulation in the cells of the plague microbe of a reserve nonspecific polysaccharide.

CONCLUSIONS

1. A difference was noted in the chemical composition of plague microbes incubated on Hottinger agar at 28 and 37°. The latter were distinguished by a considerably increased content of polysaccharide, a greater quantity of lipids and a lesser amount of glucosamine and phosphorus, mainly RNA phosphorus.

2. Galactose, glucose, xylose and ribose were detected in the polysaccharide portion of both cultures. The content of galactose in cultures incubated at 37° was many times greater than its content in cultures incubated at 28° while the quantity of glucose and ribose was approximately two times greater in cultures incubated at 28°.

3. The polysaccharide which was accumulated in considerable amounts by cells of plague microbe incubated at 37° was nonspecific.

4. Apparently there is a bond between the capability of plague microbes incubated at 37° for the accumulation of a nonspecific polysaccharide and their synthesis under these conditions of specific capsular protein.

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[The following English summary appears with the Russian article.]

A difference was noted in the chemical composition of Past. pestis cultured on Hottinger's agar at 28 and 37°C. As demonstrated, Past. pestis cells grown at 37°C differed from those grown at 28°C by a considerably increased polysaccharide content, a greater amount of lipids and a lesser amount of glucosamine and phosphorus, mainly of the RNA phosphorus. Galactose, glucose, xylose and ribose were revealed in the polysaccharide portion of both cultures. The galactose content was much greater in cultures grown at 37°C than in those grown at 28°C, whereas the glucose and ribose content was about double in cultures grown at 28°C. As established, polysaccharide, accumulated in considerable quantities by Past. pestis, grown at 37°C, was nonspecific. On the basis of the data obtained a supposition was put forward on the relationship between the capacity of Past. pestis, grown at 37°C, to nonspecific polysaccharide accumulation and the synthesis of specific capsular protein in these conditions.

Table 1

Chemical characteristics of cultures of plague microbe incubated at various temperatures.

Temperature of incubation	Content (in %) of							
	Moisture	Ash	Nitrogen	Phos-phorus	Glucos-amine	Protein	Poly-sacchar-ide	Lipids
28°	8.8	4.6	12	1.47	0.54	76.8	2.7	3.1
37°	7	3.8	10.8	1.07	0.32	67.5	12.6	5.2

Table 2

Determination of phosphorus based on fractions in cultures incubated at 28 and 37°.

Temperature of Incubation	Content of phosphorus (in %)					
	Overall	Acid-soluble	Lipid	Nucleinic acids		
				Overall	RNA	DNA
28°	1.47	0.14	0.06	1.27	0.9	0.37
37°	1.07	0.44	0.08	0.6	0.31	0.29

Table 3

Results of the quantitative determination of sugars by the chromatographic method.

Temperature of incubation	Content of sugars (in %)		
	Galactose	Glucose	Ribose
28°	0.2	0.7	0.6
37°	5.8	0.3	0.3

Table 4

Content of polysaccharide protein in unwashed and washed cells of plague microbe.

Treatment of cells	Content (in %)			
	28°		37°	
	Poly-saccharide	Protein	Poly-saccharide	Protein
Unwashed	3.8	76.2	9.22	73.1
Washed	2.7	76.8	12.6	67.5